

REMARKS

Applicants enclose the Sequence Listing for the above-captioned application and a 3.5" floppy disk containing the Sequence Listing. I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR § 1.821(c) and (e), respectively, are the same. The specification has been amended to provide SEQ ID NOS for the sequences disclosed therein. This submission contains no new matter.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: July 3, 2001



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Attorney Docket No.: 5808.200-US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Martin Lehmann

Serial No.: 09/488,265

Confirmation No: 4209

Group Art Unit: 1652

Filed: January 20, 2000

Examiner: T. Saidha

For: Improved Phytases

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Commissioner for Patents
Washington, DC 20231

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

IN THE SPECIFICATION:

The paragraph from page 17, line 16 – page 19, line 3 has been amended as follows:

Figure 1: Design of the consensus phytase-1 sequence. ~~The letters represent the amino acid residues in the one-letter code.~~ The following sequences were used for the alignment: phyA from *Aspergillus terreus* 9A-1 [Mitchell, D. B., Vogel, K., Weimann, B. J., Pasamontes, L. & van Loon, A. P. G. M. (1997) The phytase subfamily of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi *Aspergillus terreus* and *Myceliophthora thermophila*, *Microbiology* 143, 245-252]; from amino acid (aa) 27; SEQ ID NO: 1]; phyA from *A. terreus* cbs116.46 [EP 897985]. A heat resistant phytase of *Aspergillus fumigatus* with superior performance in animal experiments. Phytase optimization and natural variability. In: *The Biochemistry of phytate and phytases* (eds. Rasmussen, S.K; Raboy, V.; Dalbøge, H. and Loewus, F.; Kluwer Academic Publishers); from aa 27; SEQ ID NO: 2; phyA from *Aspergillus niger* var. *awamori* (Piddington et al (1993) *Gene* 133, 55-62; from aa 27; SEQ ID NO: 3); phyA from *A. niger* T213 (EP 897985); from aa 27; SEQ ID NO: 4); phyA from *A. niger* strain NRRL3135 [van Hartingsveldt, W., van Zeijl, C. M. F., Hartevelde, G. M., Gouka, R. J., Suykerbuyk, M. E. G., Luiten, R. G. M., van Paridon, P. A., Selten, G. C. M., Veenstra, A. E., van Gorcom, R. F. M., & van den Hondel, C. A. M. J. J. (1993) Cloning, characterization and

overexpression of the phytase-encoding gene (phyA) of *Aspergillus niger*. Gene 127, 87-94; from aa 27; SEQ ID NO: 5]; phyA from *Aspergillus fumigatus* ATCC 13073 (Pasamontes, L., Haiker, M., Wyss, M., Tessier, M. & van Loon, A. P. G. M. (1997) Cloning, purification and characterization of a heat stable phytase from the fungus *Aspergillus fumigatus*, Appl. Environ. Microbiol. 63, 1696-1700; from aa 25; SEQ ID NO: 6]; phyA from *A. fumigatus* ATCC 32722 (EP 897985); from aa 27; SEQ ID NO: 7); phyA from *A. fumigatus* ATCC 58128 (EP 897985); from aa 27; SEQ ID NO: 8); phyA from *A. fumigatus* ATCC 26906 (EP 897985); from aa 27; SEQ ID NO: 9); phyA from *A. fumigatus* ATCC 32239 (EP 897985); from aa 30; SEQ ID NO: 10; phyA from *Emericella nidulans* [Pasamontes, L., Haiker, M., Henriquez-Huecas, M., Mitchell, D. B. & van Loon, A. P. G. M. (1997a). Cloning of the phytases from *Emericella nidulans* and the thermophilic fungus *Talaromyces thermophilus*. Biochim. Biophys. Acta 1353, 217-223; from aa 25; SEQ ID NO: 11]; phyA from *Talaromyces thermophilus* (Pasamontes et al., 1997a; from aa 24; SEQ ID NO: 12); and phyA from *Myceliophthora thermophila* (Mitchell et al., 1997; from aa 19; SEQ ID NO: 13). The alignment was calculated using the program PILEUP. The location of the gaps was refined by hand. Capitalized amino acid residues in the alignment at a given position belong to the amino acid coalition that establish the consensus residue. In bold, beneath the calculated consensus sequence (SEQ ID NO: 98), the amino acid sequence of the finally constructed consensus phytase (Fcp) is shown (SEQ ID NO: 14). The gaps in the calculated consensus sequence were filled by hand according to principals stated in Example 1.

The paragraph from page 17, line 27 – page 18, line 15 has been amended as follows:

Figure 5: DNA and amino acid sequence of consensus phytase-10 (SEQ ID NO: 25, and SEQ ID NO: 26, respectively). ~~The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The amino acid sequence of the mature consensus phytase-10 is shown in SEQ ID NO:24.~~ The sequence of the oligonucleotides that were used to assemble the gene are in bold letters. The names of the respective oligonucleotides and the amino acids that differ relative to consensus phytase-1 are underlined. The fcp10 gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6, CP-7.10, CP-8.10, CP-9.10, CP-10.10, CP-11.10, CP-12.10, CP-13.10, CP-14.10, CP-15.10, CP-16.10, CP-17.10, CP18.10, CP-19.10, CP-20.10, CP-21.10, and CP-22.10. The newly synthesized oligonucleotides are additionally marked by the number 10. The phytase contains the following 32 exchanges

relative to consensus phytase-1: Y54F, E58A, D69K, D70G, A94K, N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V, A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V, R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E. The underlined mutations revealed a stabilizing effect on consensus phytase-1 when tested as single mutations in consensus phytase-1.

The paragraphs on page 27, line 11-25 have been amended as follows:

Figure 22: DNA and amino acid sequence of consensus phytase-3-thermo[11]-Q50T (SEQ ID NOS:90-91). ~~The amino acids are written below the corresponding DNA sequence using the one-letter code.~~

Figure 23: DNA and amino acid sequence of consensus phytase-3-thermo[11]-Q50T-K91A (SEQ ID NOS:92-93). ~~The amino acids are written below the corresponding DNA sequence using the one-letter code.~~

Figure 24: DNA and amino acid sequence of consensus phytase-10-thermo[5]-Q50T (SEQ ID NOS:94-95). ~~The amino acids are written below the corresponding DNA sequence using the one-letter code.~~

Figure 25: DNA and amino acid sequence of consensus phytase-10-thermo[5]-Q50T-K91A (SEQ ID NOS:96-97). ~~The amino acids are written below the corresponding DNA sequence using the one-letter code.~~